# CRISPR/CAS9 Genome Editing of Putative Cassava Flowering Genes

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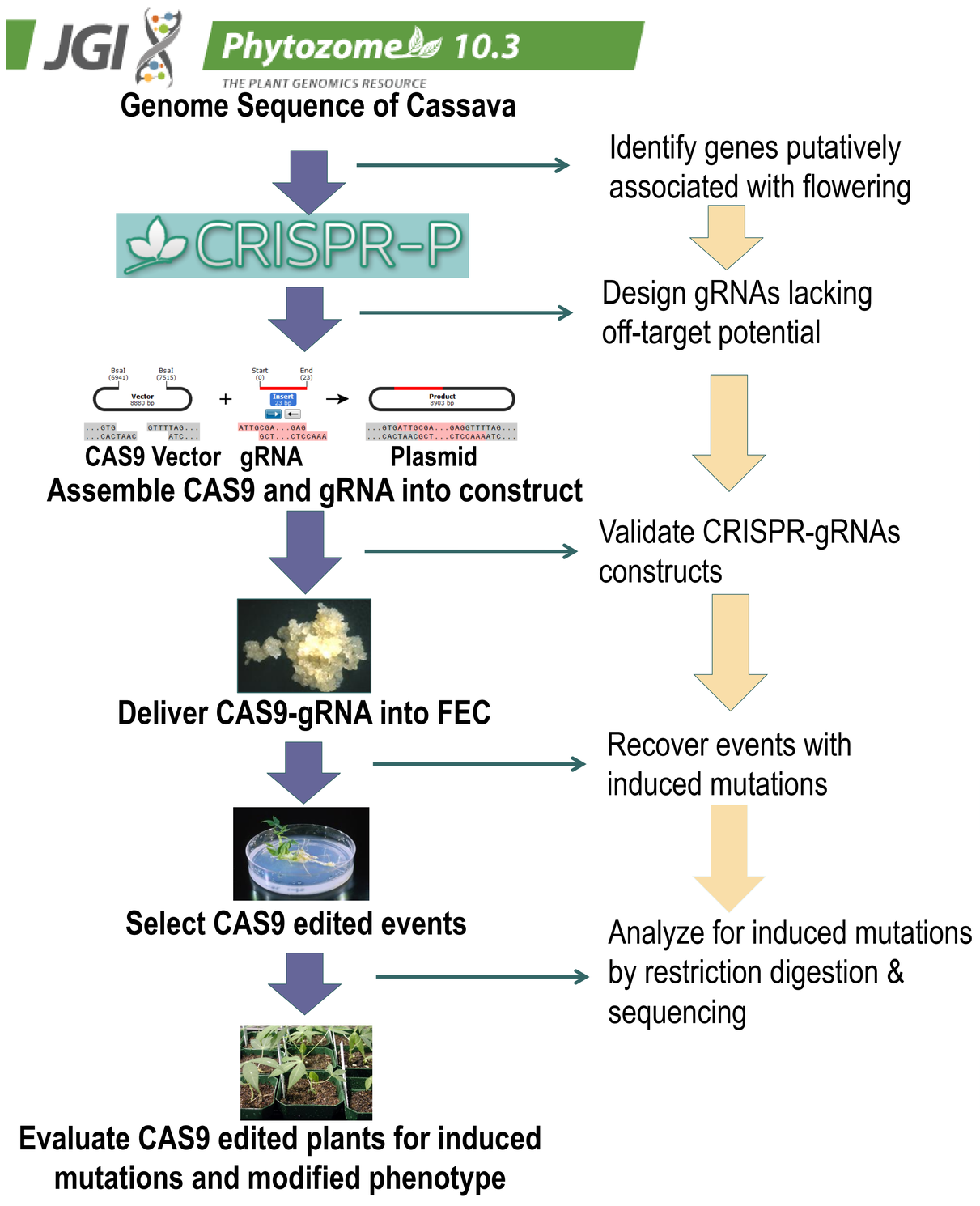
## INTRODUCTION

Cassava (*Manihot esculanta*) is an important source of food, income and industrial raw materials. Rapid breeding of elite varieties to meet increasing demand is hampered by low rates of seed set, attributed to irregular flowering habits in many genotypes. The genetic basis of flowering in cassava is poorly understood. Studies in Arabidopsis and other species have identified key pathways and genes controlling enhancement and inhibition of flowering. CRISPR/CAS9 editing techniques offer novel, reliable alternatives for validation of gene function in plants. This study seeks to employ CRISPR/CAS9 technology for targeted modification of key flowering genes in cassava. As these pathways are also implicated in root tuberisation, information gained will increase understanding of both flowering and storage root development in cassava.

## SPECIFIC OBJECTIVES

1. Identify key genes & pathways controlling flowering and storage root development in cassava
2. Design and clone CRISPR/CAS9 constructs for modifying flowering phenotypes
3. Generate and analyze CAS9 edited cassava plants for induced mutations and modified flowering phenotypes

## APPROACH




## ACCOMPLISHED ACTIVITIES

- 35 genes putatively associated with flowering identified from six gene families

| Gene | Abbreviation | No. homologs |
|---|---|---|
| Gigantae | GI | 2 |
| Constans | CO | 8 |
| Flowering locus T | FT | 10 |
| Flowering locus D | FD | 3 |
| Flowering locus C | FLC | 5 |
| Apetala 1 | AP1 | 3 |
| Suppressor of Overexpression of Constans 1 | SOC1 | 2 |
| Leafy | LFY | 2 |
| **Total** | | **35** |

- 10 gRNAs designed and cloned for three targets
- FEC produced as target tissue for gene editing and genetic transformation

## ONGOING ACTIVITIES

- Design and screen new CRISPR/CAS9 constructs
- Transform best constructs into cassava
- Analyze and evaluate CAS9 edited plants for induced mutations and modified phenotypes

## REFERENCES

**Ceballos** et al., 2015. DOI 10.1007/s00122-015-2555-4, **Adeyemo** *et al*. 2011. DOI 10.1007/s12042-011-9065-6; **Pin** P, Nilsson O. 2012. DOI: 10.1111/j.1365-3040.2012.02558.x; **Navarro** et al., 2011. *Nature* 478, 119–122; **Nekrasov** et al., 2013 DOI 10.1038/nbt.2655, http://www.phytozome.net/

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